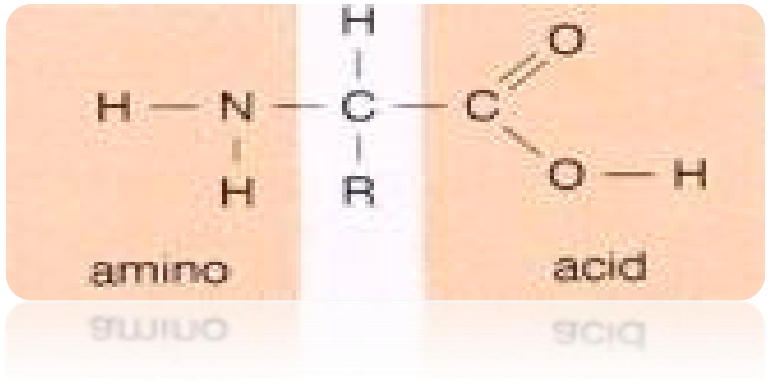
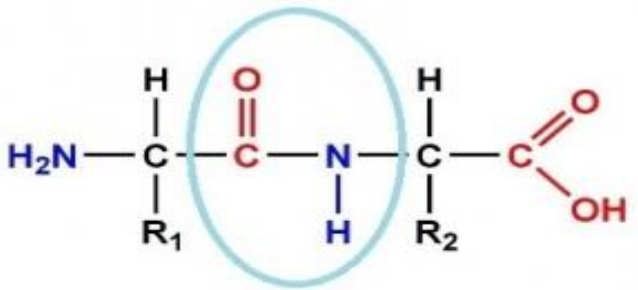
Qualitative tests of amino acids

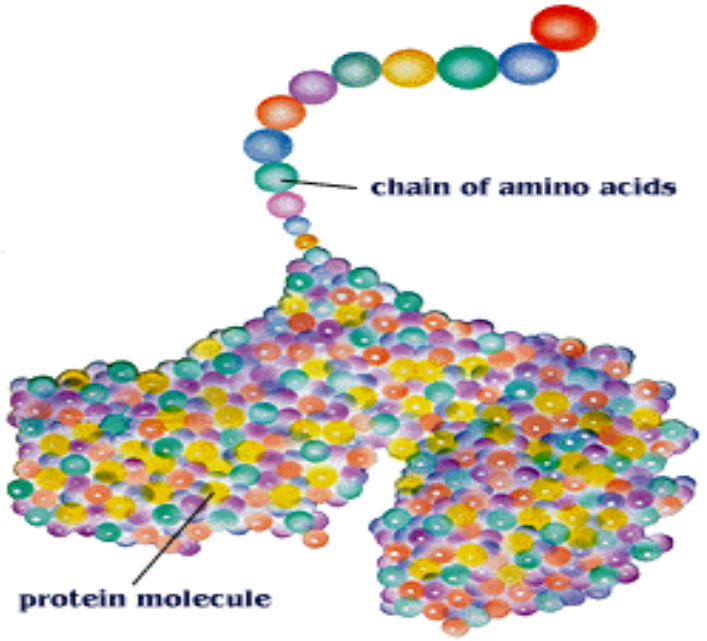


Amino acids :

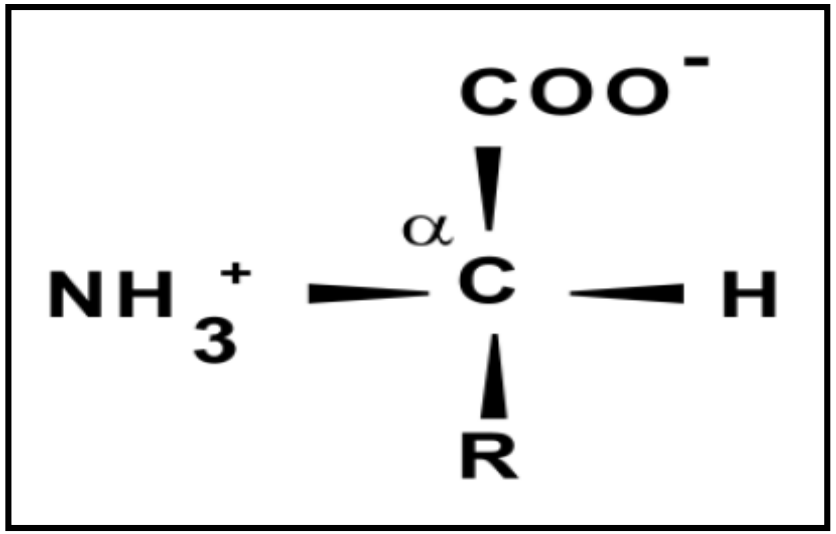
- Amino acid play A central role as building block of proteins.
- as intermediates in metabolism, converted to specialized products.
- There are **20** natural amino acids that are found within proteins.

All of them are L-α amino acids.





Amino acids structure



-All amino acids found in proteins have this basic structure, differing only in the **structure of the R-group** or the side chain.

-The simplest, and smallest, amino acid found in proteins is glycine for which the R-group is hydrogen (H).

Classification of amino acids :

Classification of amino acid depending on the R-group ionization (polarity) in water:

1- Non-polar.

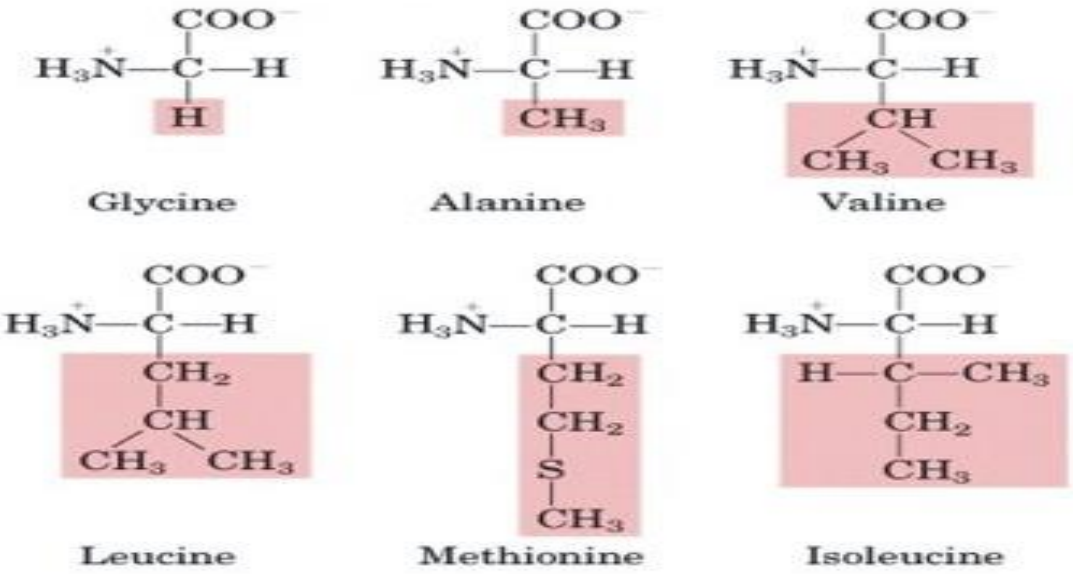
2- Uncharged polar.

3-polar amino acids :

A-Basic polar (positively charged).

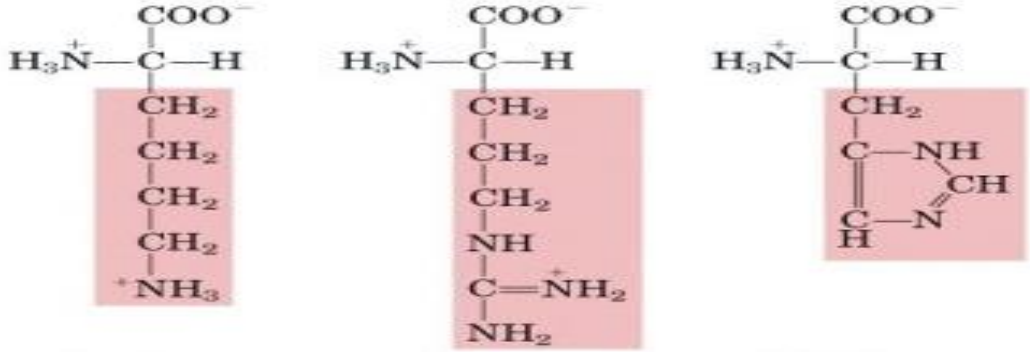
B- Acidic polar (negatively charged).

Nonpolar, aliphatic R groups

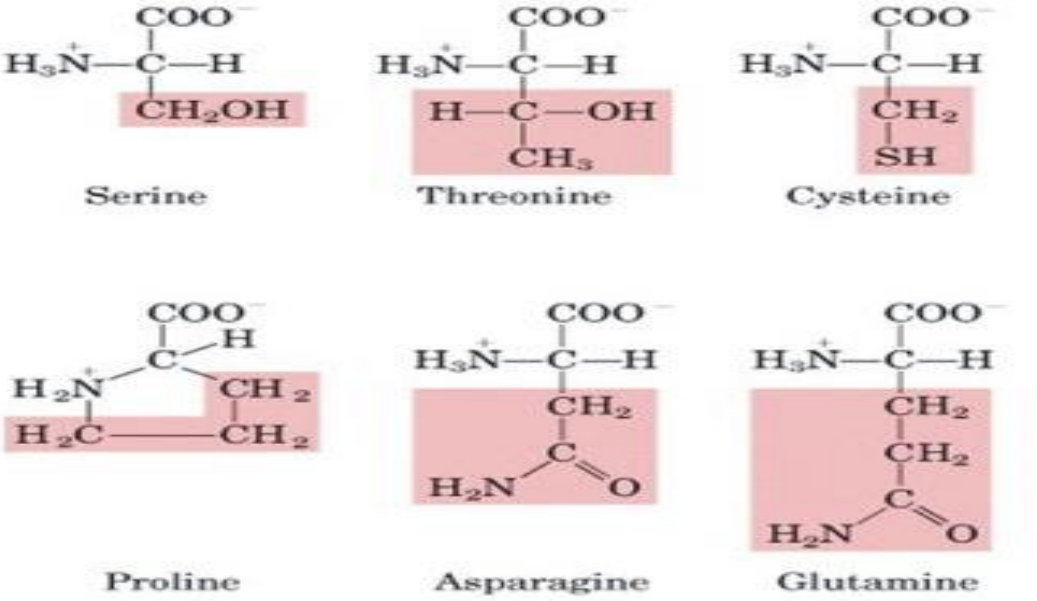


Aromatic R groups

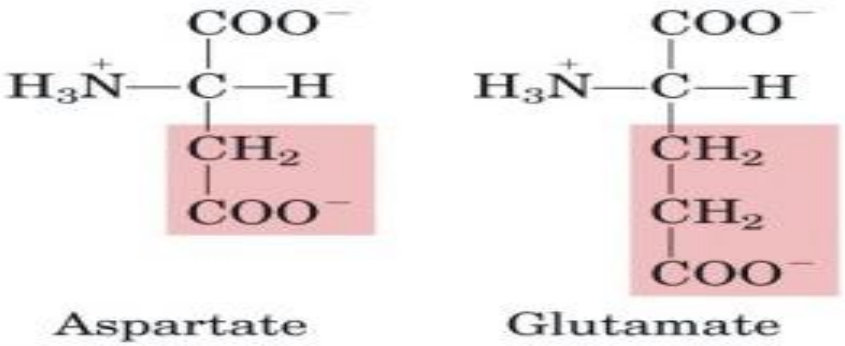
Positively charged R groups



Polar, uncharged R groups



Negatively charged R groups



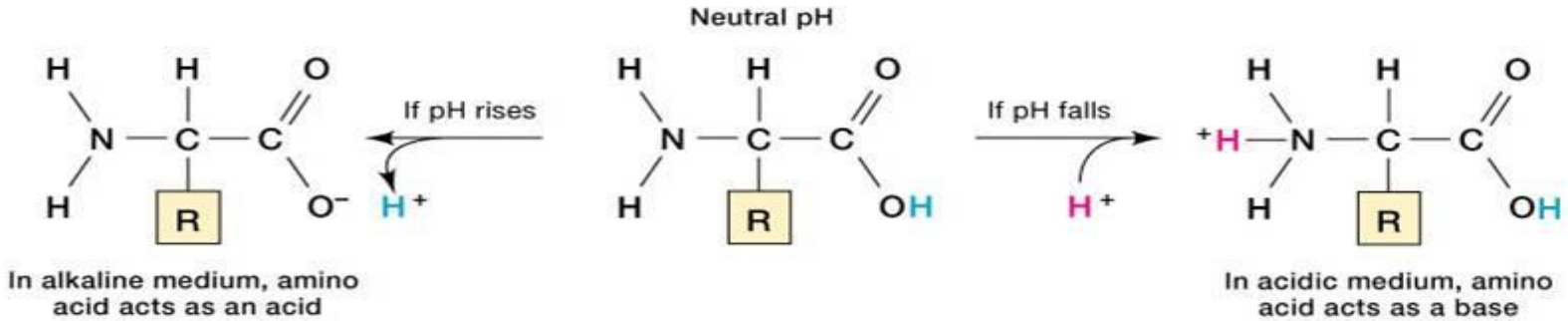
Some properties of Amino Acids :

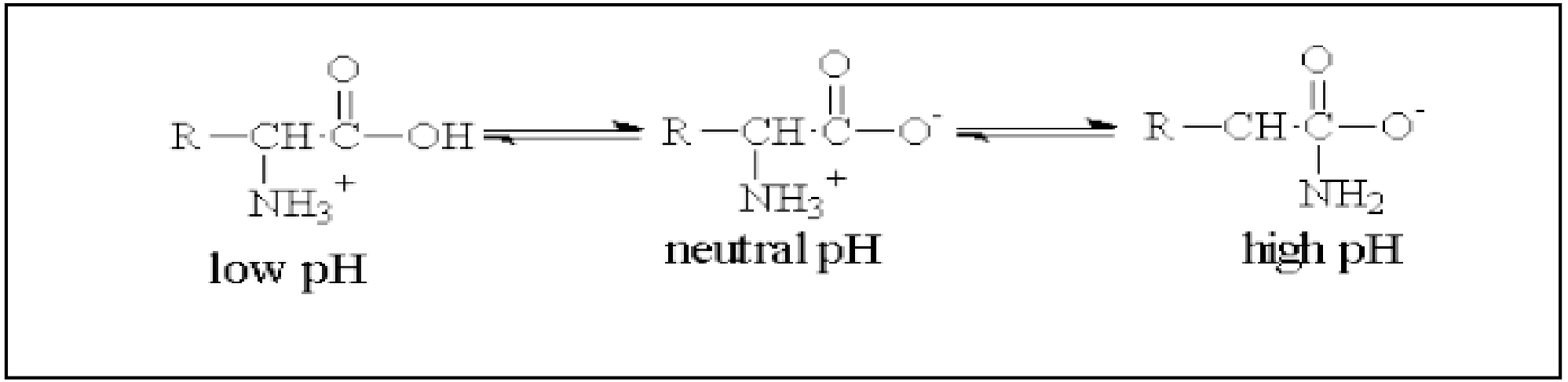
1- Amphoteric Compounds:

which mean they can **act as acids and bases**

- Due to presence of carboxyl group COOH that able to **donate** proton(H^+), and convert to COO^- (COOH → COO^-).

- Also presence of amino group NH2 which is enable to **accept** this proton(H^+) and convert into $NH3^+$ (NH2 → $NH3^+$).





Amino acids are amphoteric Compounds

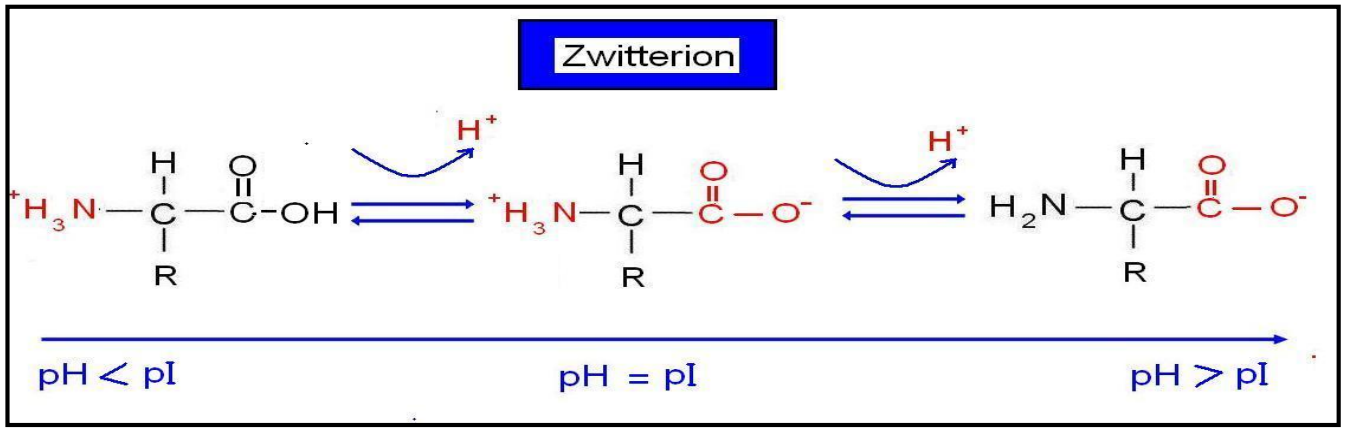
Amphoteric properties of amino acids due to the presence of their ionizable α-amino and α-carboxylic group can act sometimes as acids and sometimes as bases **depending on the pH of their media** .

2- Isoelectric point (PI):

It is the pH value at which concentration of anionic and cationic groups are equal (i.e. the net charge of this molecule equals zero).

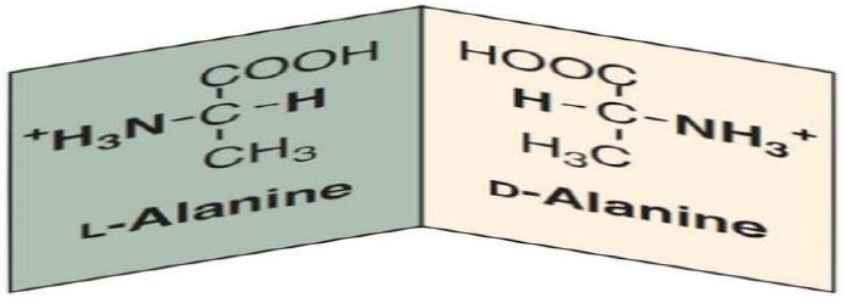
It is known as a point at which the molecule does not move to either cathode or anode if it is put in electric field and its solubility is minimum so it is possible to precipitate at this point.

Each amino acid have a different PI



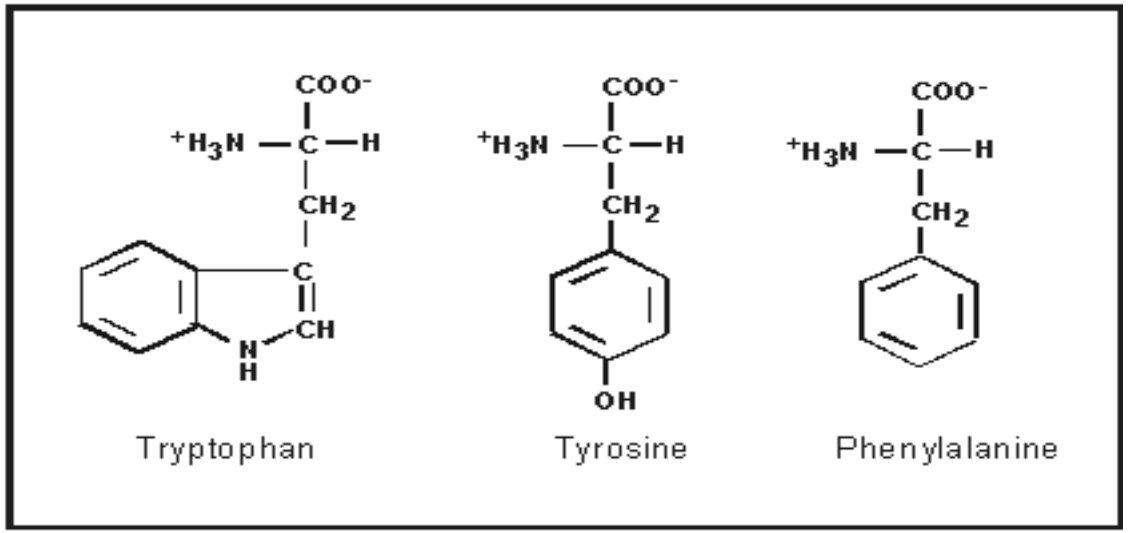
3-Optical Activity :

Amino acids are able to rotate polarized light either to the left (livo) L- a.a or to the right (dextro) D- a.a , since they have an **asymmetric C** atom (a carbon atom linked to 4 different groups), except glycine which lacks asymmetric C atom (has 2 H+ on α-C) .



4-Light Absorption:

The aromatic amino acids tryptophan , tyrosine , phenyl alanine absorb ultraviolet light at 280nm ,which explains the absorption of proteins at **280nm**.



Qualitative tests of amino acids

- 1.Solubility test
- 2. Ninhydrin tes
- 3.Xanthoproteic test
- 4. Millon's test
- 5.Sakaguchi Test
- 6. Lead sulfite test

Qualitative assays

Determine if specific substance is there or not, by color or some other quality.

Quantitative assays

Determine the concentration of a substance.

1.Solubility test:

-Objective:

investigate the solubility of selected amino acid in various solutions.

-Principle:

Polar amino acids are more soluble in water[polar] than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution.

Polar amino acids are soluble in polar solvent, and vice versa.

Method:

1.Add 4ml of different solvents in 3 clean test tubes then place 1 ml of each amino acid.

2.Shake the tubes thoroughly, then leave the solution for about one minute.

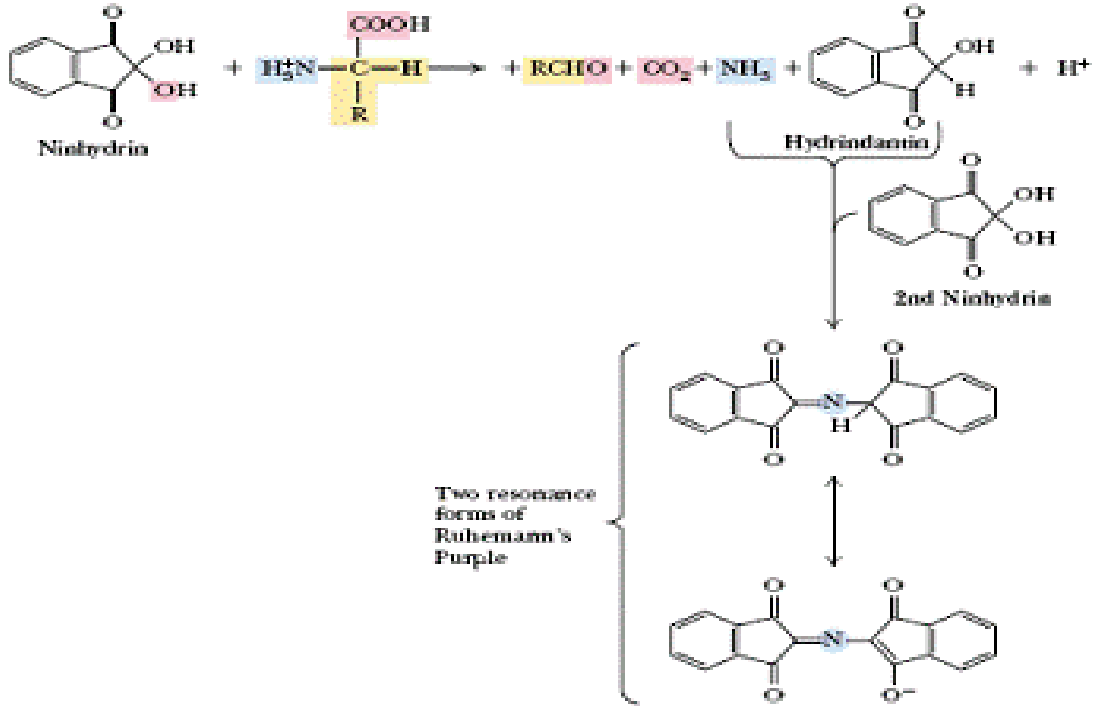
3.Notice what happened to the solution .

4. Record your result .

Result:

	glycine	Arginine
o.1M HCL		
0.1M NaOH		
Chloroform		

2. Ninhydrin test:



Objective:

-to detect α-L-amino acids

Principle:

1.Ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes (on pH range 4-8), ammonia and CO2 though a series of reactions.

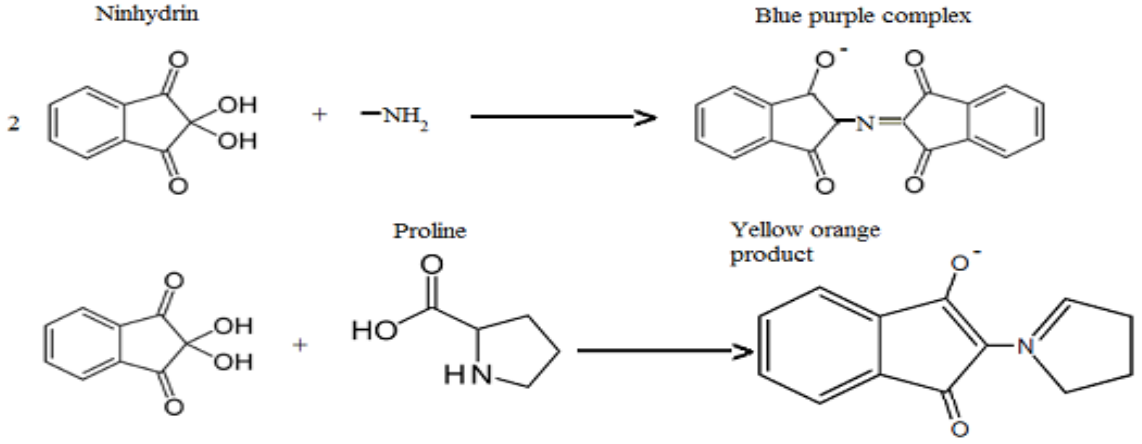
The net result is ninhydrin in a partially reduced from hydrindantin.

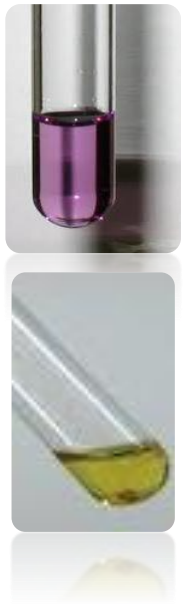
2.Ninhydrin then condenses with ammonia and hydrindantin to produce an intensely **blue or purple** pigment, sometimes called ruhemann's purple

All amino acids that have a free amino group will give positive result (**purple color**) .

While not free amino group-proline and hydroxy-proline (amino acids) will give a (**yellow color**).

Note: Many substances other than amino acids, such as amines will yield a blue color with ninhydrin, particularly if reaction is carried out on filter paper.





Method:

1-Place 1 ml of each of the solutions in a test tube and add 1 ml of ninhydrin solution.

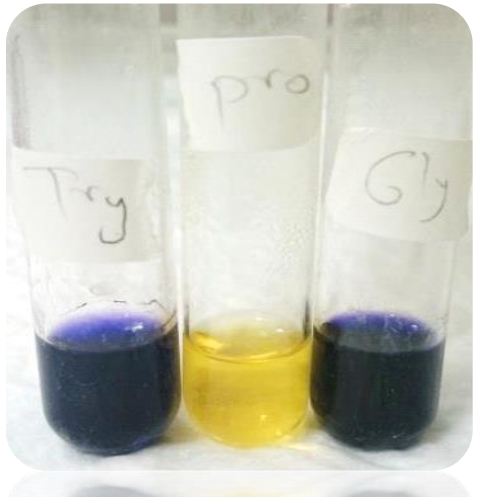
2- Boil the mixture over a water bath for 2 min.

3- Allow to cool and observe the blue color formed

4- Complete the below table.

Result:

Tube		Result	Conclusion
A	**Glycine**		
B	**Tryptophan**		
C	**Proline**		



3.Xanthoproteic test :

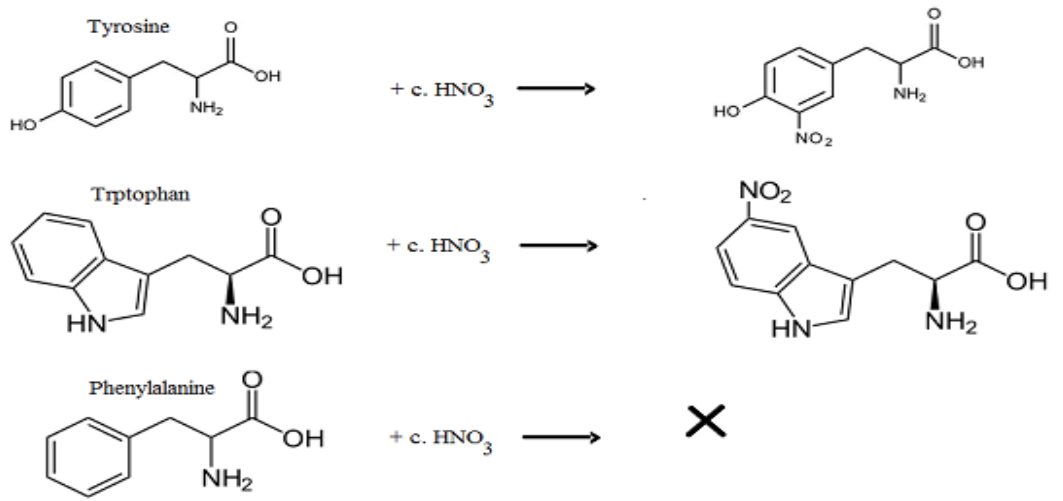
Objective:

to differentiate between aromatic amino acids which give positive results [**yellow color**] and other amino acids.

Principle:

Concentrated nitric acid react with aromatic nucleus present in the amino acid side chain [nitration reaction] → giving the solution **yellow color**.

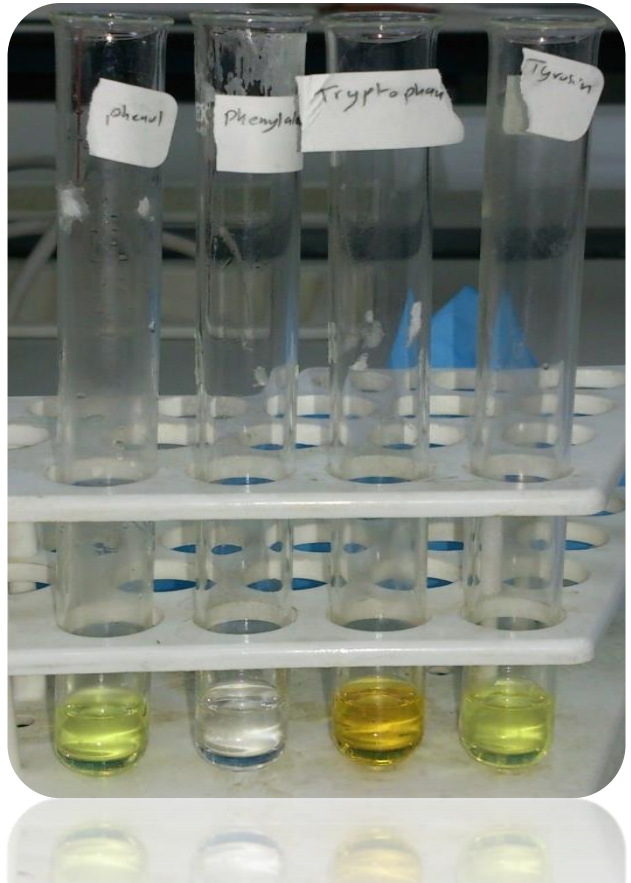
* The salts of these derivatives are orange in color.



Note:

Amino acids tyrosine and tryptophan → contain activated benzene rings [aromatic nucleus] which are easily nitrated to yellow colored compounds.

The aromatic ring of phenyl alanine **dose not react** with nitric acid despite it contains a benzene ring, but it is **not activated**, therefore it will not react



Method:

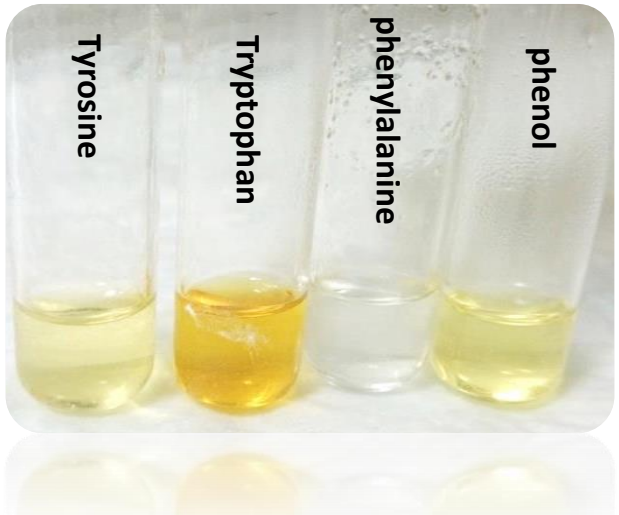
1- Label four tubes (1 - 4), then add 1 ml of each amino acid solutions and phenol solution to those test tubes each alone.

2- Add 1 ml of concentrated HNO3. then record your result

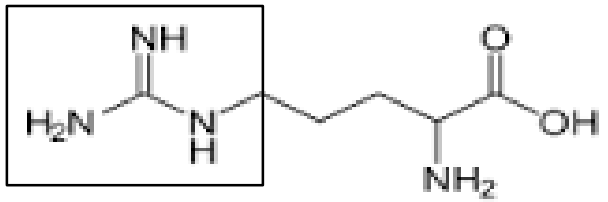
3-Now COOL THOROUGHLY under the tap and CAUTIOSLY add 5 drops of 10M NaOH to make the solution strongly alkaline(the alkaline is added to be sure about the nitration).

Result:

	+ HNO3	
Tyrosine		
Tryptophan		
plenylalnin		
Glycine		



4. Sakaguchi Test:



Objective:

detection of amino acid containing **gauanidium group**. In other words it's a test for, **arginine**.

Principle:

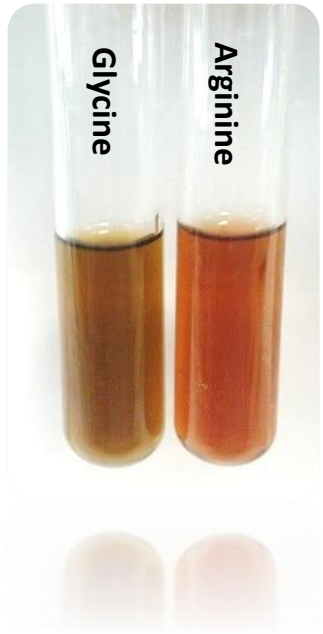
In **alkaline** solution, arginine react with α-naphthol and sodium hypobromite /chlorite as an oxidize agent, to form **red complexes** as a positive result.

Method:

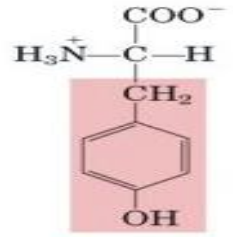
- Label 2 test tube and put in each one 2 ml of the amino acid solution .
- Add to each tube 2ml of NaOH solution. Mix well
- Add to each tube 2ml of α-naphthol solution. Mix well
- Add to each tube 5 drops of sodium hypobromite solution, and record your result

Result:

Tube	Observation	Conclusion
Glycine		
Arginine		



5.Millon's test:



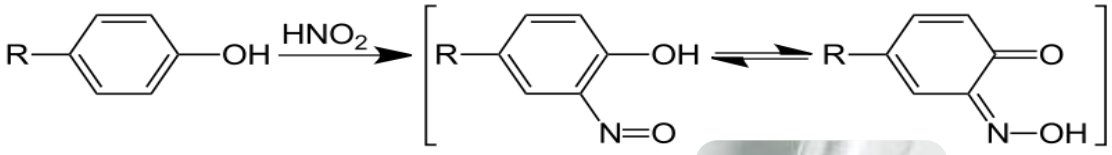
Tyrosine

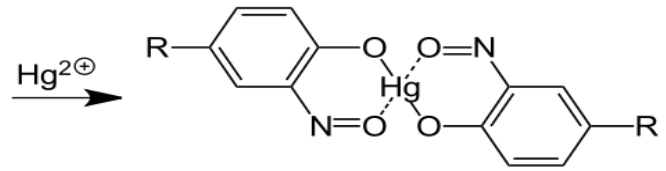
Objective:

This test is specific for tyrosine. Because it is the only amino acid containing a phenol group, a hydroxyl group attached to benzene ring.

Principle:

The phenol group of tyrosine is first **nitrated by nitric acid** in the test solution. Then the nitrated tyrosine complexes mercury ions in the solution to form a **brick-red** solution or precipitate of nitrated tyrosine, in all cases, appearance of **red color is positive test**.





Note: all phenols (compound having benzene ring and OH attached to it) give **positive** results in Millon's test

6. Lead Sulfite Test:

Objective:

This test specific for**–SH [sulfhydral group]**containing amino acid (**Cysteine**).

Principle:

- Sulfur in cystine, is converted to sodium sulfide by boiling with 40% NaOH.
- The Na2S can be detected by the precipitation of PbS (lead sulfide) from an **alkaline** solution when adding lead acetate Pb (CH3COO)2.